Echocardiographic, morphometric and biomarker changes in cats followed from 6 to 24 months of life

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Abstract max 300 words

Objectives The aim of the study was to evaluate echocardiographic, morphometric, and biomarker changes in cats followed between 6 to 24 months of age.

Methods 24 European shorthair cats in a colony were evaluated at birth for body weight (BW) and at 6, 12, 18 and 24 months of age for morphologic variables (BW, body condition score [BCS], head length [HL] and width [H]), N-terminal B-type natriuretic peptide (NT-proBNP), insulin-like growth factor-1 (IGF-1), and echocardiographic measurements.

Results BCS, HW, NT-proBNP, left ventricular free wall in diastole and left atrium diameter increased significantly until 12 months, while HL and interventricular septum in diastole (IVSd) increased significantly until 18 months, and BW and aortic diameter (Ao) increased significantly until 24 months. IGF-1 increased significantly until 12 months though decreased significantly thereafter until 18 months. There were significant associations ($R_2 \geq 0.6$) between IVSd and HL, between Ao and BW, and between IVSd and change in IGF-1 in the 6 months before the respective time point.

Conclusions and relevance Associations between body and cardiac measures have been described in adult cats and cats with cardiac hypertrophy. This study suggests comparable associations in healthy cats evaluated in early adult life; however, future studies including larger numbers of cats and more time points earlier and later in life are needed to determine any potential relationship between early growth in cats and echocardiographic measurements later in life.

Introduction

There is a possible interaction between body size and cardiac health in cats. Cats with hypertrophic cardiomyopathy (HCM) are skeletally larger (i.e., larger heads, vertebrae, and longer humeri) and heavier at diagnosis.[1-4] They are also heavier at an early age compared to cats without HCM.[5] A potential mechanism for this interaction involves insulin resistance and/or growth hormone – insulin-like growth factor (IGF)-1 axis, as binding of insulin and IGF-1 to their receptors on the cardiomyocyte stimulates myocardial protein synthesis and can cause ventricular hypertrophy.[6-8] Some, but not all studies have identified insulin resistance and elevated growth hormone or IGF-1 concentrations in cats with HCM,[2, 4, 5, 9] and cats with asymptomatic HCM can have higher body condition score (BCS), serum insulin, and circulating cardiac biomarkers.[10]

Multiple studies have identified associations between bodyweight (BW) and left ventricular measurements in healthy cats and cats with HCM.[11-18] These studies show differences in study design, with primarily intact cats that were all or mostly adults, including single or different breeds, and different gender ratios. All these studies examined the cats at one single time point, and did not report BCS, making it impossible to evaluate how many of the cats in these previous studies were ideal body weight or overweight/obese, which would identify possible confounded associations between BW and echocardiographic measurements. A previous study in cats with asymptomatic HCM showed significant associations between circulating cardiac biomarkers, echocardiography, BW, and BCS.[10]

Programming is the process of long-term effects from a positive or negative event during a sensitive or critical period of development. More specific, programming can result from early life experiences and impact the development of subsequent cardiac disease. Fetal programming has been shown in several animal and human studies, showing amongst others the effect of alterations in maternal nutrition on fetal growth and heart disease.[19] In cats, a possible relationship between

growth and cardiac measures can be extrapolated from the associations between BW and left ventricular measurements at adult age, and the possible interaction between body size and cardiac health. Although previous studies have provided information on growth in cats with HCM[5] or LVH,[2] growth was evaluated retrospectively. One study of Maine Coon cats retrospectively collected information on body weight at 6 and 12 months of age and showed that cats with HCM were larger at 6 and 12 months than cats without HCM.[5] Another study looked at the effect of growth on cardiac health at adult age.[2] Cats between 3-7 years of age in a colony were retrospectively reviewed for body weight at 6, 12, and 18 months of age, and underwent echocardiography, blood analysis and morphologic evaluation. In that study, 50% of cats had echocardiographic evidence of left ventricular hypertrophy (LVH), which was significantly associated with head width (HW), BW, N-terminal B-type natriuretic peptide (NT-proBNP), and IGF-1 concentrations. However, echocardiography was only performed at a single time point with cats at different ages. Other limitations of the study were that BW was not available for all cats until 6 months of age, BCS were not available during growth, and cats ate a variety of diets during growth and throughout adulthood. A prospective study evaluating BW, skeletal size, BCS, and echocardiographic measurements would be a next step in better understanding the relationship between cardiac measures and body size from young age through adulthood. Therefore, the objective of this study was to prospectively evaluate changes in echocardiographic measures, morphologic variables, and circulating blood marker during the first two years of life in cats.

Materials and Methods

Twenty-four female European shorthair cats from 11 different litters (1-5 cats per litter) were included in the study. These cats were participating in a separate observational nutritional study from birth until 24 months of age, where the maximum sample size was defined as 24 cats. Furthermore, the sample size of 24 cats was suitable for both the capacity of the research facility to guarantee animal welfare and to ensure that the sampling workload could be conducted in reliable conditions by one person in order to avoid manipulation bias. Cats were habituated to human contact and manipulation between birth and 6 months of age, and all cats were group-housed in a colony in compliance with EU regulations and were fed *ad libitum*. Cats were fed a growth/reproduction diet (Royal Canin Mother and Babycat, Royal Canin SAS) from birth until weaning, a growth diet (Royal Canin Kitten, Royal Canin SAS) from weaning until 10.5 months of age, and a commercial adult diet (Royal Canin Neutered Young Male, Royal Canin SAS) from 10.5-24 months of age. All cats were neutered at 8 months of age. Data from morphometric measurements, echocardiography, and blood sampling were obtained at time points 6 months, 12 months, 18 months, and 24 months. Body weight at birth was also recorded. Measurements were performed in conscious cats with no sedation. Morphometric measures included BW, BCS (9-point scale) and head length (HL), and HW. HL and HW were measured according to previously described techniques.[4] Head measurements and BCS were performed by the same person at each time point. All cats underwent physical examination and echocardiography (two-dimensional [2-D], M-mode, and color flow Doppler echocardiography (GE Vivid 7 Dimension, General Electric Systems), performed by a single board-certified veterinary cardiologist [DJC]) using a 7.5 mHz probe on Harmonic mode-octave at the highest frame rate available. Cats were scanned from beneath while in right lateral recumbence to obtain 2-D and M-mode images from right parasternal views. Loops were recorded of the right parasternal long-axis four-chamber view, the right

parasternal long-axis left ventricular (LV) outflow ('5-chamber') view, the right parasternal short-axis view at the level of the papillary muscles, and the right parasternal short-axis view at the level of the aortic valve. M-mode images were guided from 2-D images of the right parasternal short-axis view at the level of the papillary muscles.[20]

Measurements were made from recorded images. All LV wall thickness measurements were made from either 2-D or M-mode images. 2-D maximal LV wall thickness was measured on the first frame after mitral valve closure on the long axis four- and five-chamber view or at the frame with the largest end-diastolic left ventricular internal diameter in diastole (LVIDd) in the short axis view at the level of the papillary muscles. A leading-edge-to-leading-edge method of measurement was used, being careful to exclude the pericardium, false tendons, or papillary muscles. M-mode measurements were taken in a right parasternal short-axis view at the level of the papillary muscles using the leading-edge to leading-edge method.[20] At least three measurements were made of the thickest region identified for each view of the end-diastolic interventricular septum (IVSd) and left ventricular free wall (LVWd), recording the largest repeatable value. All cats were assessed for focal wall hypertrophy from the right parasternal long-axis inflow and outflow views.

The size of the left atrium (LA) was assessed using two separate methods: using 2-D images from a right parasternal short-axis view to calculate the ratio of diastolic LA diameter to aortic root (Ao) diameter (LA:Ao) measured on the first frame after aortic valve closing and using a right parasternal long-axis four-chamber view to measure the diameter of the LA measured parallel with the mitral annulus in the last frame before mitral valve opening.[3] At least three measurements were made of each variable, recording an average value for each. The presence or absence of systolic anterior motion of the mitral valve was assessed on a 2-D right parasternal long-axis LV outflow view, using cine loop played back at reduced speed and by visualization of characteristic colour

Doppler flow.[20-22]Simultaneous electrocardiographic monitoring was not possible due to cat compliance.[23] Unsedated blood pressure was measured by a single veterinarian in a quiet environment using Doppler technique, using the mean value of three separate measurements.

Blood was collected after food restriction for approximately 10 hours at each time point for NT-proBNP and IGF-1. EDTA plasma was collected at specified time points and stored at -20°C for batch analysis. Analyses for IGF-1 (IGF-1 RIA CT, Mediagnost) and NT-proBNP (Feline CardioPet NT-proBNP, IDEXX Laboratories) were performed by commercial laboratories.

Statistical methods

Linear Mixed models were used for modelling the effect of Time (6, 12, 18 and 24 months) on the echocardiographic measurements (2-D-LVIDd, 2-D-IVSd, 2-D-LVWd, Ao, LA, LA:Ao, M-IVSd, M-LVIDd and M-LVWd), morphologic variables (BW, BCS, HL and HW) and blood markers (IGF-1 and NT-proBNP). NT-proBNP data were log transformed for respecting model assumptions (normally distributed residuals and homoscedasticity). Tukey HSD was applied for multiple comparisons between time points and the level of significance was set at 0.05% for two-sided tests. In order to evaluate the association between echocardiographic measurements and morphologic and biomarker variables, a linear mixed model was developed for each of the echocardiographic measurements as dependent variable and morphologic variables and blood markers as independent variables. Time and its interaction with other independent variables were also modelled as fixed effects. Cat factor was modelled as random term. Both directions stepwise linear mixed model regression was then applied in order to select most relevant morphologic and biomarkers variables and avoid multicollinearity.

Associations between echocardiographic variables and the evolution of independent variables during the previous 6 months were then evaluated. Independent variables were transformed into the difference over a 6-month period (6 to 12 months, 12 to 18 months and 18 to 24 months) and combined respectively with dependent variables at 12, 18 and 24 months. Linear mixed models were then developed for each of the echocardiographic measurements as dependent variable and Time (12, 18 and 24 months) and evolutions on 6 months periods of morphologic and biomarkers variables (6 to 12 months, 12 to 18 months and 18 to 24 months) as independent variables. Time and its interaction with other independent variables were also modelled as fixed effects. Cat factor was modelled as random term. Both directions stepwise linear mixed model regression was then applied in order to select most relevant morphologic and biomarkers variables and avoid multicollinearity.

Results were obtained in RStudio Version 1.1(www.rstudio.com, RStudio Inc). Linear mixed models were calculated from the *lme4* function of *LmerTest* package [24] and the function *step* from the same package was used for the stepwise regression. Tukey HSD was applied from *emmeans* function from *emmeans* package (https://CRAN.R-project.org/package=emmeans, R package version 1.4.1.). Results are expressed as median and range (minimum, maximum).

Results

Median birthweight was 0.125 kilograms, ranging from 0.100 to 0.170 kilograms. All 24 cats completed the follow up between 6 and 24 months of age. Changes over time in BW, BCS, HL, HW, IGF-1, and NT-proBNP are described in Table 1. BW continuously increased significantly between 6 and 24 months. Body condition score and HW increased significantly until 12 months; however, HL increased significantly until 18 months. The prevalence of cats that were overweight or obese (i.e., >5/9 BCS) was 38% at 6 months, 79% at 12 months, 88% at 18 months, and 88% at 24 months.

None of the cats had evidence of structural heart disease on echocardiography at any time point, and there was no identification of focal wall hypertrophy. Changes in echocardiographic measurements over time are shown in Table 2. 2-D-LVWd, LA-max, M-mode LVIDd, and M-mode LVWd increased significantly until 12 months; however, 2-D-IVSd increased significantly until 18 months and Ao diameter increased significantly until 24 months. Median (range) of heart rate at the different time points was 178 (148-240) at 6 months, 152 (120-176) at 12 months, 152 (112-180) at 18 months, and 160 (128-200) at 24 months of age. Median (range) of blood pressure (mmHg) at the different time points was 122 (102-143) at 6 months, 148 (118-163) at 12 months, 156 (130-180) at 18 months, and 153 (112-207) at 24 months of age. Blood pressure was ≥180 mmHg in 1 cat at 18 months of age and 2 cats at 24 months of age. No cat had a cardiac murmur at the age of 6 and 12 months, 1 cat had a murmur (I/VI) at 18 months but not at 24 months, and 2 cats had a murmur (I/VI) at 24 months of age. No cat had a gallop rhythm at 6 months, 1 cat had a gallop rhythm at 12 months but not at 18 months, and 1 cat had a gallop rhythm at 24 months of age.

NT-proBNP decreased significantly between 6 and 12 months but did not change significantly thereafter. Two of the 24 cats had an NT-proBNP concentrations >100 pmol/L (<100

pmol/L is considered unlikely to have heart disease).[25] One of these was at 6 months of age (117 pmol/L) and the other was from a separate cat at 24 months (122 pmol/L). The cat with the elevated value at 24 months had an intermittent grade I/VI cardiac murmur auscultated at that time but no other cardiac abnormalities were noted for either cat. Other causes for elevated NT-proBNP could not be identified in either cat.

Nineteen of the 24 cats had IGF-1 concentrations >350 ng/mL (the upper reference value for healthy cats established by the lab analyzing the samples) at 6, 12, 18, or 24 months of age, with 5 cats having IGF-1 concentrations > 665 ng/mL and 1 cat with IGF-1 concentration between 800 and 1000 ng/mL,[26] all at 12 months of age. IGF-1 increased significantly between 6 and 12 months and then decreased significantly between 12 and 18 months.

Table 3 shows the associations between dependent variables (2-D-LVIDd, 2-D-IVSd, 2-D-LVWd, Ao, LA, LA:Ao, M-IVSd, M-LVIDd and M-LVWd) and the independent variables time, morphologic variables (BW, BCS, HL and HW) and blood markers (IGF-1 and log transformed NT-proBNP) which were selected by stepwise regression. Interaction between time and morphologic variables or blood markers are not presented because none of them were selected from stepwise regression. There is a significant impact of time on 2-D-LVIDd, 2-D-IVSd, 2-D-LVWd. Those echocardiographic measurements are also significantly associated with HW, HL and both BW and HW respectively. Aortic diameter and LA were significantly associated with BW with no impact of time. LA:Ao, M- LVIDd and M-IVSd were only significantly associated with time. Only dependent variable M-LVWd was significantly associated to both a morphologic variable (HL) and blood marker variable (NT-proBNP). Moreover, there was no impact of time on this measurement. Overall, echocardiographic measurements were more frequent associated with morphologic variables (BW, HL, and HW but not BCS) than with blood markers. Associations were strongest between 2-D-IVSd and HL (R_2 = 0.58), and between Ao and BW (R_2 = 0.58).

Table 4 shows the statistically significant associations between dependent variables and evolution of morphologic and biomarkers in the previous 6 months (Table 4). There was a significant impact of time on 2-D-LVIDd and M-IVSD, Ao and 2D-IVSD. 2-D-LVIDd and M-IVSD were associated with none of morphologic and biomarkers variable evolution. There was an association between Ao and BW and 2D-IVSD was associated with both a morphologic marker (HL) and a biomarker (IGF-1). 2-D-LVWd was as well associated with both a morphologic marker (HL) and biomarkers (NT-proBNP and IGF-1), and LA:Ao with IGF-1 but with no impact of time. In contrast to the associations at separate time points, echocardiographic measurements were more frequent associated with changes in blood markers than changes in morphologic variables during time periods.

Discussion

The objective of this study was to prospectively evaluate changes in echocardiographic measures, morphologic variables and blood markers in healthy cats from 6 months to 24 months of age. Results showed significant changes over time for both dependent and independent variables, as well as associations between dependent variables and independent variables, the latter both expressed as absolute measure as well as change over 6 months' time. This was to investigate whether echocardiographic measures are associated to morphologic variables and blood markers at a specific time point, but also to investigate whether echocardiographic measures are associated to changes in these morphologic variables and blood markers, i.e. measured IGF-1 concentration but also an increase in IGF-1 would be associated with an echocardiographic measure.

The 2D-IVSd was associated with HL, and also to changes in HL in the previous 6 months. Comparable findings of echocardiographic measurements associated with measures of head size have been described in cats with cardiac pathologies. One previous study that excluded Maine coon cats[4] identified an association between hypertrophic cardiomyopathy (HCM) and HW and HL, and a study in 28 cats of varying breeds (including 4 Maine coon cats) only showed an association between LVH and HW.[2] The results of this study contributes to the hypothesis of a relationship between cardiac and body size, not only in cats with cardiac pathologies but also in healthy cats. Because cats in the current study were only followed until 24 months of age and none of the cats had developed cardiac pathologies at that age, it is unclear whether this association in these young cats has a predictive value for development of cardiac pathology later in life.

2-D-IVSd was also significantly associated with the change in IGF-1 in the 6 months before that time point. Two previous studies in cats showed a significant association between IGF-1 and LVH[2] or HCM,[5] and the results described here contribute to the general understanding of the relationship between cardiac measures and body size and the mechanism behind this relationship.

The change in IGF-1 concentration between separate time points, but not the IGF-1 concentration at time points itself, was associated with LA:Ao, 2-D-IVSd and 2-D-LVWd. It can be hypothesized that an increasing IGF-1 concentration and/or variation in IGF-1 concentration have an influence on cardiac measures. To the authors' knowledge, no studies have reported IGF-1 concentrations during growth in cats. However, in humans, serum IGF-1 concentrations increase during growth, with peak values at puberty.[27] In the current study, the highest mean IGF-1 concentration occurred at 12 months of age. None of the cats showed signs of hypersomatotropism[28], therefor acromegaly was not suspected in these cats.

The other variable associated with measures of left ventricular thickness was NT-proBNP. The M-LVWd was associated with NT-proBNP, and 2-D-LVWd was also associated to changes in NT-proBNP in the 6 months before to that time point. It is important to note, however, that there were only 2 cats that had NT-proBNP concentrations >100 pmol/L at any time point, and they were without cardiac abnormalities or an identified cause for elevated NT-proBNP. Previously, NT-proBNP showed associations with measures of cardiac size in cats with LVH[2] or HCM,[29, 30] though the results in the current study suggest there might be a comparable association in healthy cats as well. NT-proBNP is secreted from cardiac myocytes during cardiac myocyte stretch, pressure overload, and neurohormonal stimuli,[29] which are all processes that may intermittently occur during cardiac growth. While other studies of NT-proBNP have included at least some cats <2 yrs of age,[30-32] none have reported NT-proBNP concentrations for healthy cats during the first 2 years of life.

There was no significant association between BCS and any echocardiographic measurement, though it should be noted that 88% of cats in the current study were overweight by the time they were 18 months of age, likely due to the cats being fed *ad libitum* since birth. Therefore, the weight of a cat at 18 or 24 months of age does not necessarily reflect the cat's body

size and cardiac measures. However, BW was associated with 2-D-LVWd, Ao and LA, and changes in BW were associated with Ao. Previous studies have shown comparable associations between BW and left ventricular measures in healthy cats,[11, 13, 16-18] though the study described here is the first to examine cats at different time points in life and including BCS. The associations between BW and 2-D-LVWd, Ao and LA suggest that healthy larger cats simply have larger hearts, however if the reason for the association between BW and left ventricular measurements was merely the result of larger cats having larger, thicker hearts, one would expect that BW in adulthood would be associated with all echocardiographic measures and not only measures of the left ventricle. Also, variables that were associated with measures of left ventricular thickness in the current study (i.e., BW, head size, NT-proBNP, and IGF-1) have been associated with LVH or HCM in previous studies.[2, 4, 5] The association between obesity and left ventricular hypertrophy has been described in humans,[33] dogs,[34] and cats,[5] though it is still unclear whether this also exists for cats with healthy cardiac function.

One notable finding from the current study was wide variation in cats' BW, growth rates, and BCS (Table 1) even though the cats had identical housing, handling, and were all fed the same diet *ad libitum*. This may be due to genetic factors since cats were from 11 different litters or to individual variability, although the sample size was too small to evaluate these factors in more detail. In addition, while male cats are predisposed to HCM, all cats in the current study were female so their risk may have been lower than in the general population. Studying the role of early growth and nutrition on the heart in a controlled situation is advantageous although results would need to be confirmed in a home environment and in cats of different breeds and gender.

There are important limitations to the current study. Most importantly, cats were only studied until 2 years of age so it is not known if any of these cats will develop HCM or LVH later in life. Longer longitudinal studies are needed to determine the relationship between early growth

and the development of HCM or other cardiac pathologies over the course of cats' lifetimes. It can be hypothesized whether the results observed can be due to variability in obtaining ultrasound images and performing measurements on them. Intra-observer variability of echocardiographic measurements was investigated by Chetboul et al. [35], showing that increased experience of the observer decreases the coefficient of variation of within- and between-day repeated measurements. The board-certified veterinary cardiologist [DJC]) has a longtime experience in performing echocardiography in cats, thereby limiting possible influence of variability on the results. The results of this study also may not be generalizable to pet cats, given that these were of a single breed from 11 litters and were housed in a colony situation, with a controlled environment. However, the feeding situation is not unlike that in many households where cats are fed *ad libitum*. In one study of Maine Coon cats, the percentage of cats fed *ad libitum* was 89% during growth and 90% as adults.[5] Evaluations did not begin until 6 months of age so very early differences in growth may have been missed and should be considered for evaluation in future studies. Nonetheless, although cats are clinically considered to reach maturity by 1 year of age, physeal closure of some long bones in the cat does not occur until as late as 25 months of age, so some growth is possible after 1 year. In fact, bones of the skull may fuse even later with sphenoid, frontal, parietal, and temporal bone not fusing until 2-4 years of age,[36] which could explain the increased head size between 12 and 18 months of age. In the current study, body weight continued to increase until the 24 months' time point which could be partially due to continued growth. Body condition score increased only until 12 months of age, therefore the increase in bodyweight due to development of obesity is less likely. Despite the evaluations starting only at the age of 6 months, this study is still the first to describe echocardiographic measurements in cats repeatedly examined at different time points in early-adult life. Only one measure of skeletal size (i.e., head size, as assessed by HL and HW) was used.[4] Other studies have looked at humerus length or vertebral size which may provide

useful information since it is likely to be less influenced by breed than head size.[4, 5] In addition, cats in the current study were fed a single diet which was changed at 3 time points during the study. Diet has been described to alter echocardiographic measurements in cats with HCM[37] and rodent models of cardiomyopathy[38, 39], therefore this also could have influenced the results of the study. Different diets or even keeping the cats on the same diet for the duration of the study may have yielded different results. A variety of other factors could have influenced the results, including genetic and epigenetic influences, as well as behavioral factors that could influence food intake and, therefore, growth. A final limitation is the relatively small sample size which limited the number of multivariable comparisons that could be made. Larger studies could help to identify other potential associations with the outcome variables.

Conclusions

Associations between body and cardiac size have been described in adult cats and cats with cardiac hypertrophy. This study suggests comparable associations in healthy cats evaluated in early adult life, however future studies including a larger number of cats and more time points earlier in life are needed to determine any potential relationship between early growth in cats and echocardiographic measurements as indicators of development of heart disease or cardiac hypertrophy later in life.

Tables

Table 1. Morphologic variables and blood markers at 6, 12, 18 and 24 months of age in 24 healthy cats. Data are presented as median (range). Different superscript letters identify within-group comparison (P <0.05).

	6 months	12 months	18 months	24 months	Effect of time
BW (kg)	2.7 (2.1-3.4)^{a}	3.6 (2.6-4.5)^{b}	4.5 (3.3-5.8)^{c}	4.3 (3.0-5.4)^{d}	<0.0001
BCS (1-9)	5 (5-6)^{a}	6 (5-8)^{b}	7 (5-8)^{b}	6 (5-8)^{b}	<0.0001
HL (cm)	90.65 (84.20-96.80)^{a}	99.81 (93.38-106.70)^{b}	101.99 (92.91-108.57)^{c}	101.08 (91.31-109.96)^{bc}	<0.0001
HW (cm)	58.30 (55.8-62.1)^{a}	67.20 (64.37-70.86)^{b}	68.07 (62.67-72.01)^{b}	68.26 (63.21-71.89)^{b}	<0.0001
IGF-1 (ng/mL)	260.7 (103.6-424.1)^{a}	452.7 (152.1-923.7)^{b}	372.1 (166.3-630.8)^{c}	343 (116-531)^{ac}	<0.0001
NT-proBNP (pmol/L)	44 (24-117)^{a}	33 (24-78)^{b}	25 (24-77)^{b}	24 (24-122)^{b}	<0.0001

BW, body weight; BCS, body condition score; HL, head length; HW, head width; IGF-1, insulin-like growth factor-1; NT-proBNP, N-terminal B-type natriuretic peptide

Table 2 - Echocardiographic measurements (in mm) at 6, 12, 18, and 24 months of age in 24 healthy cats. Data are presented as median (range). Different subscript letters identify within-group comparison (P <0.05).

	6 months	12 months	18 months	24 months	Effect of time
2-D-LVIDd	13.5 (11.5-17.1)$_a$	14.6 (12.8-17.3)$_b$	10.0 (12.1-17.0)$_{ab}$	14.4 (12.1-16.4)$_{ab}$	0.001
2-D-IVSd	4.0 (3.2-5.0)$_a$	4.3 (3.1-5.2)$_b$	4.8 (3.4-5.9)$_c$	4.5 (3.5-5.4)$_{bc}$	<0.0001
2-D-LVWd	3.8 (3.0-4.4)$_a$	4.3 (3.4-4.9)$_b$	4.3 (3.3-4.9)$_b$	4.2 (3.4-4.7)$_b$	<0.0001
Aorta (short axis)	7.9 (7.2-9.2)$_a$	8.5 (7.6-9.6)$_b$	8.7 (7.6-10.1)$_{bc}$	8.9 (7.8-10.2)$_c$	<0.0001
Left atrium (short axis)	10.8 (8.8-12.4)$_a$	11.4 (10.3-13.6)$_b$	11.8 (10.1-13.5)$_b$	11.8 (10.1-13.5)$_b$	0.0002
Lefta atrium : Aorta	1.3 (1.1-1.6)$_a$	1.4 (1.2-1.5)$_a$	1.3 (1.1-1.5)$_a$	1.3 (1.1-1.5)$_a$	0.7564
M-LVIDd	13.4 (9.2-14.7)$_a$	14.5 (12.8-18.1)$_b$	13.6 (11.3-16.9)$_{ab}$	14.5 (11.8-17.0)$_b$	0.0001

M-IVSd	4.0 (2.7-4.8)[a]	4.7 (3.2-5.5)[b]	4.9 (3.8-5.9)[b]	4.8 (3.7-5.8)[b]	<0.0001
M-LVWd	4.0 (2.8-5.1)[a]	4.4 (3.1-5.1)[b]	4.4 (3.3-5.4)[b]	4.4 (3.1-5.7)[b]	0.0003

2-D-LVIDd, 2-D-mode end-diastolic left ventricular internal diameter in diastole; 2-D-IVSd, 2-D-mode end-diastolic interventricular septum in diastole; 2-D-LVWd, 2-D-mode end-diastolic left ventricular free wall in diastole; M-LVIDd, M-mode end-diastolic left ventricular internal diameter in diastole; M-IVSd, M-mode end-diastolic interventricular septum in diastole; M-LVWd, M- mode end-diastolic left ventricular free wall in diastole.

Table 3 - Associations between dependent variables (2-D-LVIDd, 2-D-IVSd, 2-D-LVWd, Ao, LA, LA:Ao, M-IVSd, M-LVIDd and M-LVWd) and the independent morphologic variables (BW, BCS, HL and HW), blood markers (IGF-1 and log transformed NT-proBNP) and time in 24 healthy cats.

R_2 : Coefficient of determination of the model. Independent variables selected by stepwise regression are presented. For abbreviations, see Table 2 legend.

	Body weight	Body condition score	Head length	Head width	NT-proBNP (log transformed)	IGF-1	Time	R_2
LVIDd	-	-	-	0.0211	-	-	0.0285	0.44
IVSd	-	-	0.0293	-	-	-	0.0012	0.58
LVWd	0.0057	-	-	0.0190	-	-	0.0016	0.47
Ao (short axis)	<0.0001	-	-	-	-	-	-	0.58
LA (short axis)	<0.0001	-	-	-	-	-	-	0.27
LA:Ao	-	-	-	-	-	-	0.0328	NA
M-LVIDd	-	-	-	-	-	-	0.0001	NA

M-IVSd	-	-	-	-	-	-	0.0001	NA
M-LVWd	-	-	<0.00 01	-	0.0318	-	-	0.47

Table 4 Significant associations between dependent variables (2-D-LVIDd, 2-D-IVSd, 2-D-LVWd, Ao, LA, LA:Ao, M-IVSd, M-LVIDd and M-LVWd) at 12, 18 and 24 months and evolution of independent morphologic variables (BW, BCS, HL and HW), blood markers (IGF-1 and log transformed NT-proBNP) in 24 healthy cats in the previous 6 months

R_2 : Coefficient of determination for the significant associations other than Time alone. Independent variables selected by stepwise regression are presented. For abbreviations, see Table 2 legend.

	Bodyweight	Body condition score	Head length	Head width	NT-proBNP (log transformed)	IGF-1	Time	R_2
2-D-LVIDd	-	-	-	-	-	-	0.0240	NA
2-D-IVSd	-	-	0.0037	-	-	0.0062	0.0005	0.60
2-D-LVWd	-	-	0.0246	-	0.0206	0.0119	-	0.34
Ao (short axis)	0.0390	-	-	-	-	-	0.0029	0.47
LA (short axis)	-	-	-	-	-	-	-	-
LA:Ao	-	-	-	-	-	0.0297	-	0.30
M-LVIDd	-	-	-	-	-	-	-	-

M-IVSd	-	-	-	-	-	-	0.0411	NA
M-LVWd	-	-	-	-	-	-	-	-

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Conflict of interest

Dr. Freeman has received research funding or provided sponsored lectures or consulting services for Royal Canin, Nestlé Purina PetCare, Aratana Therapeutics, and Hill's Pet Nutrition Incorporated, and serves on an Advisory Council for Aratana Therapeutics. Drs van Hoek and Laxalde are employees of Royal Canin SAS. John and David?

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Ethical approval

The study was approved by the Royal Canin Ethics Committee and the Animal Use and Care Advisory Committee of Pays de la Loire (France), reference 01934.01.

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